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Increased Genetic Instability of the Common Fragile Site at 3p14 after Integration of Exogenous DNA

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Summary

We determined previously that the selectable marker pSV2neo is preferentially inserted into chromosomal fragile sites in human × hamster hybrid cells in the presence of an agent (aphidicolin) that induces fragile-site expression. In contrast, cells transfected without fragile-site induction showed an essentially random integration pattern. To determine whether the integration of marker DNA at fragile sites affects the frequency of fragile-site expression, the parental hybrid and three transfectants (two with pSV2neo integrated at the fragile site at 3p14.2 [FRA3B] and specific hamster fragile sites [chromosome 1, bands q26-31, or mar2, bands q11-13] and one with pSV2neo integrated at sites that are not fragile sites) were treated with aphidicolin. After 24 h the two cell lines with plasmid integration at FRA3B showed structural rearrangements at that site; these rearrangements accounted for 43%–67% of the total deletions and translocations observed. Structural rearrangments were not observed in the parental cell line. After 5 d aphidicolin treatment, the observed excess in frequency of structural rearrangements at FRA3B in the cell lines with pSV2neo integration at 3p14 over that in the two lines without FRA3B integration was less dramatic, but nonetheless significant. Fluorescent in situ hybridization (FISH) analysis of these cells, using a biotin-labeled pSV2neo probe, showed results consistent with the gross rearrangements detected cytogenetically in the lines with FRA3B integration; however, the pSV2neo sequences were frequently deleted concomitantly with translocations. In the hamster fragile sites, a significantly greater rate of chromosome aberrations (gaps, breaks, and rearrangements) was seen only at the bands containing integrated pSV2neo sequences; the hamster fragile sites that did not contain integrated pSV2neo had levels of chromosome aberrations similar to that of the parental line. The cell line with pSV2neo that was integrated randomly also demonstrated a level of chromosome aberrations equivalent to that of the parental line. These results show that integration of marker DNA into FRA3B and hamster fragile sites perturbs these sites, leading to enhanced genetic instability in these regions.

Introduction

Fragile sites are chromosomal loci which show gaps or breaks when cells are exposed to specific conditions, e.g., the presence of certain chemical agents or low folic acid levels (reviewed in Sutherland and Hecht 1985). The majority (87) of recognized fragile sites are common in the human population, whereas a smaller group (26) are rare (Sutherland and Ledbetter 1989). With the exception of the fragile X at band q27.3 (FRAXA), which causes a common form of mental retardation, no pathological role has been shown for any fragile site. However, correlations between fragile sites, oncogene loci, and the breakpoints of chromosome rearrangements in cancer have generated a keen research interest in these regions over the past several years (Le Beau and Rowley 1984; Yunis and Soreng 1984; reviewed in Laird et al. 1987).

The genetic and molecular basis for chromosomal fragility at these sites is not known, nor is anything definitively known about the mechanisms of fragile-site expression. The common fragile sites are only

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weakly induced by conditions of thymidine stress, which induces the majority of the rare fragile sites. On the other hand, aphidicolin, a specific inhibitor of DNA polymerase-α, strongly induces the common fragile sites (Glover et al. 1984). Whatever the causative aspects of DNA structure or function may be, they are likely to be physiologically important, since fragile sites are evolutionarily conserved (Yunis and Soreng 1984; Miro et al. 1987). Expression of fragile sites requires induction during a preceding S phase, and suppression is achieved by agents that reduce constraints on DNA synthesis, e.g., by maintaining normal levels of thymidine or folic acid (Sutherland 1977). Most, if not all, agents known to induce fragile sites interfere with replication fork progression. This has led to the hypothesis that the gaps and breaks at fragile sites are due to failure of replication at sites that are unusually sensitive to interference during DNA synthesis (reviewed by Laird et al. 1987). Fragile sites may also be recombinogenic, as evidenced by the higher than expected frequencies of mitotic sister-chromatid exchanges (Glover and Stein 1987). Moreover, several authors have also demonstrated high levels of inter- and intrachromosomal rearrangements at fragile sites in vitro (Warren et al. 1987; Yunis et al. 1987; Glover and Stein 1988).

We determined previously the specific site of pSV2neo integration in clonal transfectants of the H3-4 hybrid line (transfected with and without aphidicolin treatment) by fluorescent in situ hybridization (FISH) using biotinylated pSV2neo. There were one to three integration sites in each clone. In 4 of the 13 cell clones transfected with aphidicolin treatment, pSV2neo was localized to 3p14, the locus of a common fragile site that is induced by this agent (FRA3B). Specific integration into two hamster chromosomes was also observed. pSV2neo was localized to hamster chromosome 1, bands q26-31, in nine clones and to hamster mar2, bands q11-13, in four clones. A total of 31 cell clones transfected without aphidicolin treatment (from three different transfection experiments), however, showed an apparently random pattern of pSV2neo integration (Rassool et al. 1991). In the present report, we present data suggesting that integration of marker DNA at known fragile sites can lead to genomic instability as evidenced by a significantly increased frequency of chromosome aberrations. Such sites of instability in the genome may be important to take into account when targeting genes and expressing genes in transgenic mice.

Material and Methods

Cell Lines

The Chinese hamster ovary (CHO) × human somatic cell hybrid, H3-4, containing a rearranged human chromosome 3, der(3)(pter→q21::q26.2→qter), as the only human component, was provided by Dr. Harry Drabkin (fig. 1*A*). As reported elsewhere, H3-4 cells exposed to aphidicolin (an agent that induces expression of the FRA3B), as well as control cells without aphidicolin treatment, were transfected with the 5.7-kb vector, pSV2neo (Southern and Berg 1982), containing the neomycin-resistance gene, using a modification of the calcium-phosphate precipitation technique described by Chen and Okayama (1987) (Rassool et al. 1991). Stable transfectants were selected and maintained in G418-containing medium throughout the experiments described. Three transfectant cell clones were selected for additional studies. Two cell clones (TI6d and TII6b) were from cultures transfected in the presence of aphidicolin, and one cell clone (TII4d) was from a culture that was transfected without fragile-site induction. One cell clone, TII6b, had pSV2neo integration at band p14 of the human der(3) and at bands q11-13 of hamster mar2 (fig. 1*C*). The other clone, TI6d, had pSV2neo integration at band p14 of human der(3), and at bands q26–31 of hamster chromosome 1 (fig. 1*D*). The transfectant TII4d had pSV2neo integrated into hamster chromosome 8 (band q13), chromosome 9 (band p15), and mar2 (bands q21-23), bands that do not contain fragile sites recognized at present (Rassool et al. (1991).

Fluorescent In Situ Hybridization

The procedure used for FISH (Rowley et al. 1990) is a modification of the method described by Lichter et al. (1990). Probes were prepared by nick-translation using Bio-11-dUTP (Enzo Diagnostics) or digoxigenin-11-dUTP (Boehringer-Mannheim Biochemicals). Hybridization of biotin-labeled probes was detected with fluorescein isothiocyanate (FITC)–conjugated avidin. Digoxigenin-labeled probes were detected by incubation with rhodamine-conjugated sheep anti-digoxigenin antibodies (Boehringer-Mannheim Biochemicals). Metaphase chromosomes were identified by DAPI staining. pSV2neo, total placental DNA (Sigma), and a 2-kb α-satellite fragment, specific for the centromeric region of chromosome 3, D3Z1 (ONCOR), were used for FISH analysis.

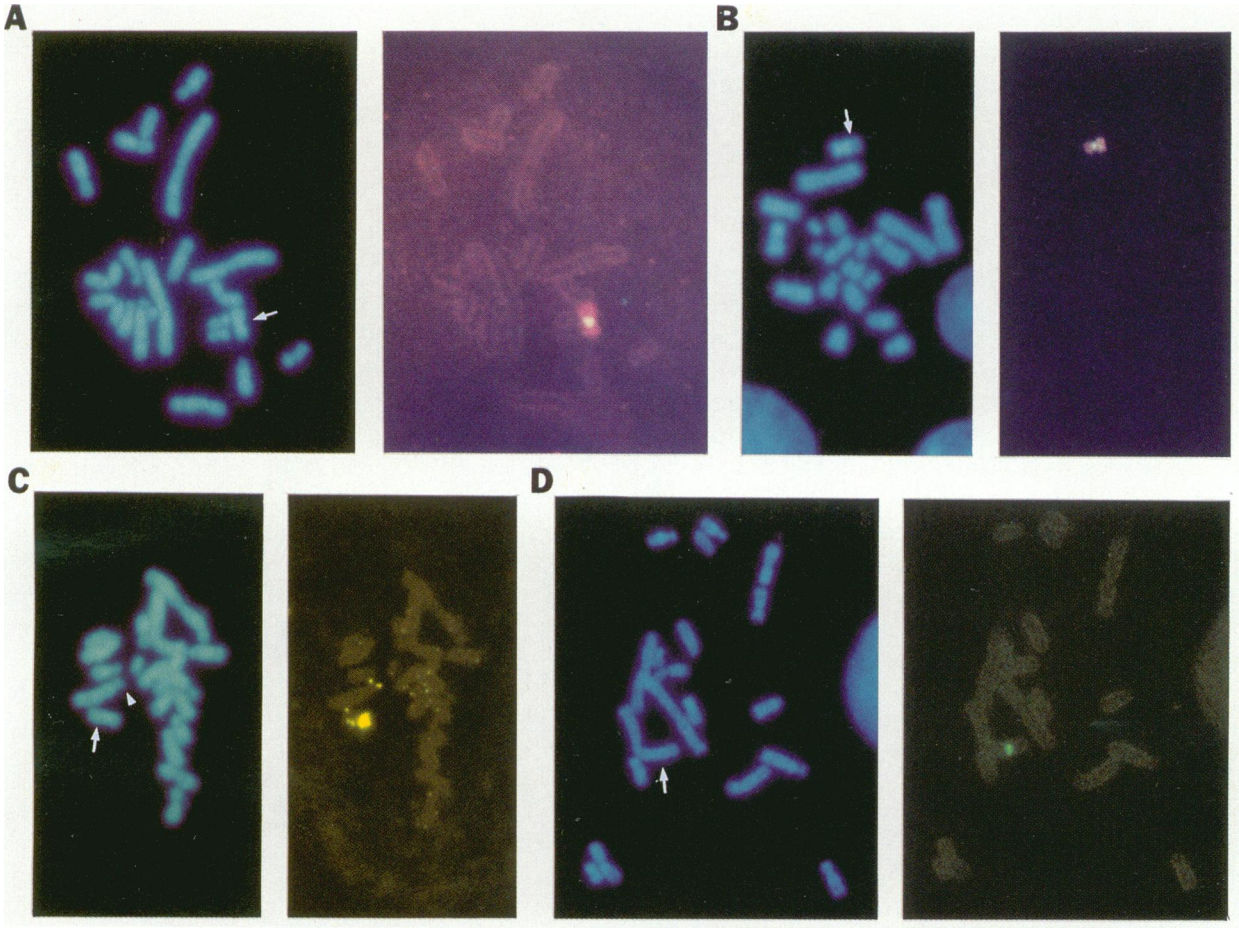

Figure 1 FISH of H3-4 cells and transfected cell lines. In each panel, the cell on the left is counterstained with DAPI; detection of hybridized probes is shown on the right. *A*, FISH of total human placental DNA and a chromosome 3 α-satellite centromere-specific probe to H3-4 metaphase cells. Digoxigenin-labeled human placental DNA, detected with anti-digoxigenin-rhodamine, identifies a single human chromosome in H3-4 cells (*red signal*). This chromosome was unequivocally identified as human chromosome 3 by cohybridization of the biotin-labeled chromosome 3–specific probe detected with FITC-conjugated avidin (*yellow signal at the centromere*). *B*, FISH of total human placental DNA to H3-4 metaphase cells after 5 d aphidicolin treatment. Hybridization of digoxigenin-labeled human placental DNA and biotin-labeled chromosome 3 centromeric probe identifies human chromosome 3 with a break at band p14 and a translocation of unidentified hamster chromosome material. The breakpoint of the unbalanced translocation on the rearranged chromosome 3 in the DAPI-stained cell is indicated (*arrow*). *C*, FISH of pSV2neo and chromosome 3 centromere probes to H3-4 cells transfected with pSV2neo after aphidicolin treatment (i.e., cell line TII6b). The chromosome 3 α-satellite probe hybridizes to the centromere of the human der(3), and the pSV2neo probe hybridizes to both chromatids at band p14 (*arrow and yellow dots*). Hybridization of pSV2neo was also detected at band p13 of a rearranged hamster chromosome t(5;?) (p13;?) (*arrowhead*). *D*, FISH of a biotin-labeled pSV2neo probe to transfectant cell line TI6d. The probe hybridizes to both chromatids of the long arm of hamster chromosome 1, bands q26–31. Hybridization to band p14 of the human der(3) was not evident in this particular cell.

Induction of Breakage and Recombination at Fragile Sites

Expression of FRA3B in the parental hybrid and in three transfectant cell lines was induced as follows: Cells were seeded at 5×10^5/10-cm plate in folic acid–deficient minimal essential medium (GIBCO) and were treated with aphidicolin (0.4 μM; Sigma) for 24 h at 37°C. Metaphase cells were prepared using standard techniques, and the cells were trypsin-Giemsa banded. Twenty-five metaphase cells were scored (for each cell line) for the presence of chromosome aberrations (gaps, breaks, translocations, and deletions).

Recombinations and deletions were induced as fol-

lows: Hybrid cells were seeded at a density of 1 × 10^5/10-cm plate and were grown in the presence of 0.4 μM aphidicolin in folic acid–deficient minimal essential medium for 5 d. Thereafter, the cells were subcultured by seeding at 1 × 10^5/10-cm plate in medium without drugs and were incubated for 24 h to allow the cells to "recover." Cells were then seeded at 10^3 cells/10-cm plate, and well separated colonies (>15 d old), consisting of >100 cells, were isolated with metal cloning cylinders (Reid 1979). Individual cell clones were expanded for further analysis. Twenty colonies (cell clones) were isolated from each of the four cell lines (H3-4, TI6d, TII6b, and TII4b). Chromosome rearrangements at FRA3B were detected by both trypsin-Giemsa banding analysis and FISH. In the derived cell clones where deletions of pSV2neo had occurred at the FRA3B, pSV2neo was retained at the other site of integration and therefore was selectable in G418. All statistical comparisons of chromosome aberrations were done by the Fisher exact test.

Results

Fragile-Site Induction

Human chromosomes.—To determine whether pSV2-neo integration at chromosomal fragile sites affected the frequency of fragile-site expression at these sites, we examined the parental hybrid cell line H3-4 and three transfectant cell lines exposed to aphidicolin for 24 h. Chromosomal aberrations were observed in the human der(3) in all four cell lines, indicating that the fragile site at band p14 of chromosome 3 is induced under these culture conditions (fig. 2). However, structural rearrangements involving 3p14 were observed with substantial frequency in the TI6d and TII6b lines (fig. 2*C* and *D*) but were not seen in either the parental line of TII4d (fig. 2*A* and *B*). Thus, in TI6d, 13 (28%) of 43 chromosome aberrations occurred at band p14 of the human der(3); 50% and 67% of total deletions and translocations, respectively, occurred at FRA3B (fig. 3*A*). In TII6b, 12

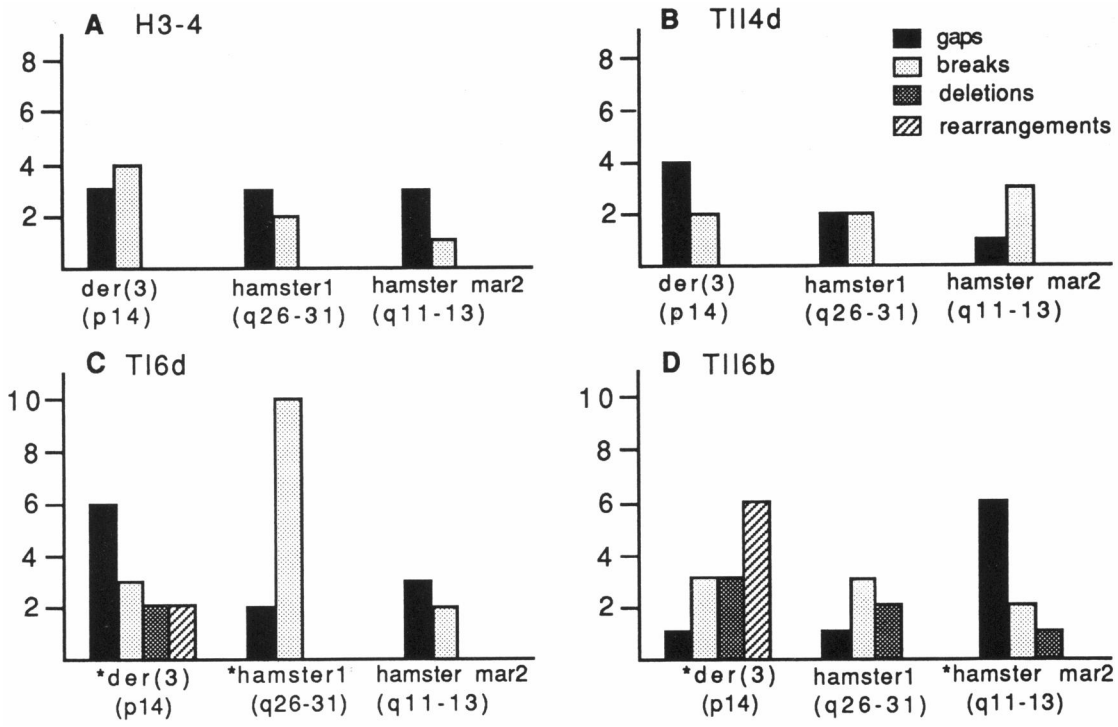

Figure 2 Bar graph showing the number and distribution of chromosome aberrations in the parental H3-4 hybrid line (*A*) and in three derived cell lines—TII4d (*B*), TI6d (*C*), and TII6b (*D*)—transfected with pSV2neo after 24 h aphidicolin treatment. The vertical bars indicate the number of gaps (■), breaks (▩), deletions (▩), and translocations (▨) observed at der (3)(p14), hamster chromosome 1 (q26–31), and hamster mar2 (q11–13), in each cell line. The chromosomes containing integration of pSV2neo in the TI6d and TII6b lines are indicated by asterisks.

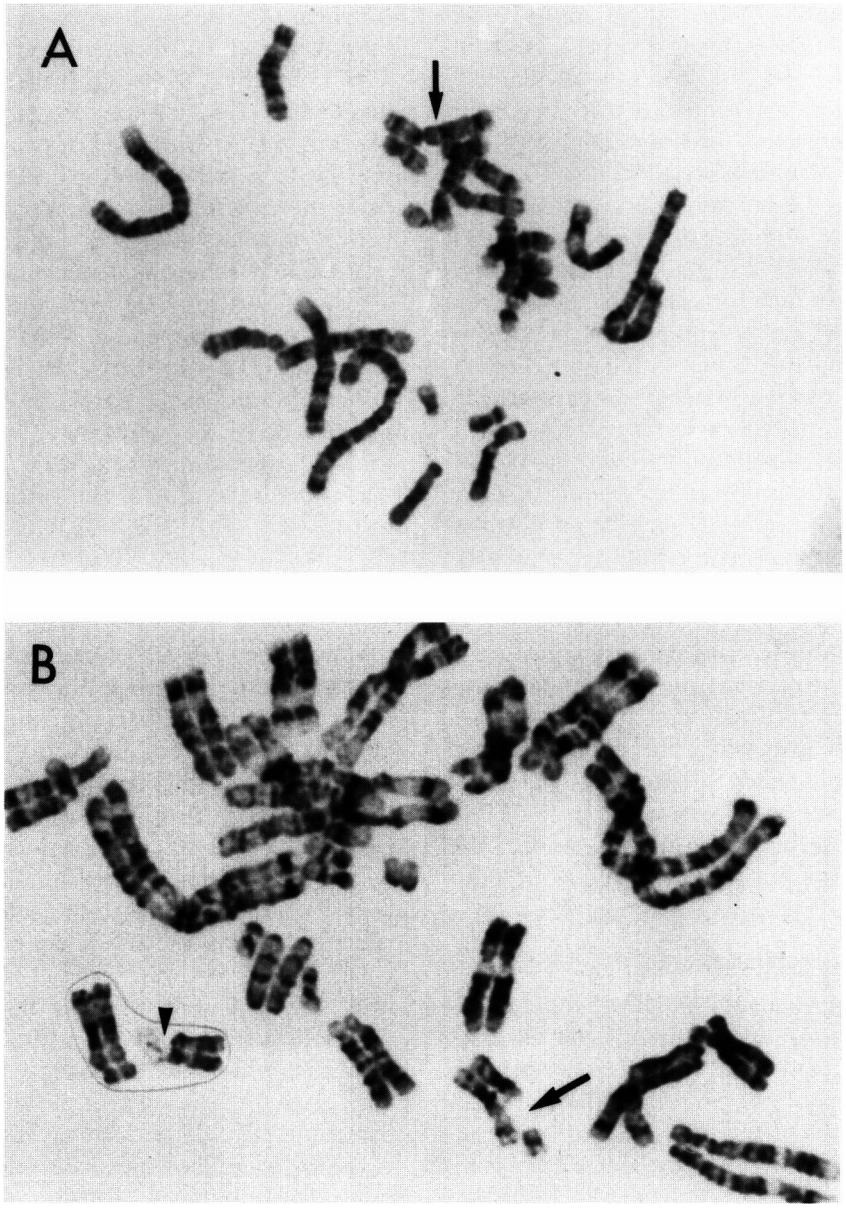

Figure 3 *A*, Trypsin-Giemsa-banded metaphase cell from the transfectant line TI6d after 24 h of aphidicolin treatment, showing an unbalanced translocation between the human der(3) with a breakpoint at band p14 and a hamster chromosome. *B*, Trypsin-Giemsa-banded metaphase cell from the hybrid cell line H3-4, showing chromosomal breaks in the human der(3) at band p14 (*arrow*) and in hamster chromosome 8 at band p17 (*arrowhead*).

(29%) of 46 chromosome aberrations were at 3p14; of the total deletions and translocations, 43% and 67% respectively, occurred at the FRA3B.

Only chromosome breaks and gaps were observed in the parental cell line, H3-4 (fig. 2*A*). Of 36 chromosome aberrations, 7 (19%) occurred at band p14 of the human der(3). In the transfectant line TII4d, 6 (18%) of 34 chromosome aberrations occurred at FRA3B (fig. 2*B*). Thus, there was no significant difference, in the frequency or type of chromosome aberrations, between this line and the parental line ($P = 1$). In TII4d, no chromosomal aberrations were observed involving the sites of pSV2neo integration (hamster chromosomes 8q13, 9p15, and mar2q21-23).

Hamster chromosomes. — Of the two previously identified aphidicolin-induced hamster fragile sites (band q26-31 of chromosome 1 and band q11-13 of mar2), chromosome aberrations at these sites occurred with a high frequency only in cell lines containing pSV2neo sequences integrated at these sites. In TI6d, 12 (28%) of 43 chromosome aberrations occurred at bands q26-31 of hamster chromosome 1, and five occurred at bands q11-13 in mar 2 (fig. 2*C*). Thus, in TI6d, which contained pSV2neo at this site (1q26-31), there was a significant increase (compared with the parental line) in chromosome aberrations at hamster chromosome 1 ($P < .003$) but not in mar2 (compare fig. 2*A* with fig. 2*C*). In the TII6b transfectant cell line, which contained pSV2neo integrated in mar2 (q11-13), chromosome aberrations in mar 2 were more frequent (compare figs. 2*A* and 2*C* with fig. 2*D*). In this line, 6 (13%) of 46 chromosome aberrations occurred at hamster chromosome 1, and 9 (20%) occurred at bands q11-13 in mar2 (fig. 2*D*).

The frequency of breaks and gaps at the two hamster fragile sites in the parental line and TII4d line was similar (figs. 2*A* and *B*). In the parental H3-4 hybrid line, 5 (14%) of 36 chromosome aberrations occurred at bands q26-31 of hamster chromosome 1, and 4 of 36 occurred at bands q11-13 in mar2. In the TII4d line, 4 (11%) of 34 chromosome aberrations occurred at hamster chromosome 1 (q26-31), and 4 (11%) of 34 occurred in mar2 (q11-13).

Induction of Deletions and Translocations

To determine the frequency of deletions and translocations involving the human der(3), the cell lines were treated with aphidicolin for 5 d, and 20 individual cell clones were isolated from each line. Chromosome rearrangements of the human der(3) were detected in the derived cell clones by both FISH, using total human placental DNA and a 2-kb α-satellite fragment specific for the centromeric region of chromosome 3, and cytogenetic analysis using trypsin-Giemsa banding. Although aberrations involved various bands of the human der(3), the overwhelming majority of deletions and rearrangements occurred specifically at band p14 (table 1). Cell clones derived from the two transfectant lines with integration of vector sequences at the FRA3B showed a substantial increase in chromosomal rearrangements at that site. Thus, in TI6d and TII6b, 10 of 20 and 11 of 20 cell clones, respectively, had deletions and translocations involving 3p14 (fig. 1*B*). In the TII6b cell line, all of the aberrations of the der(3) chromosome were observed at 3p14. By comparison, both the parental hybrid and the transfectant TII4d had fewer clones with rearrangements at 3p14 (i.e., five and seven clones, respectively).

Localization of pSV2neo in Transfectants after Aphidicolin Treatment

Since the level of rearrangements at 3p14 detectable by cytogenetic analysis was significantly elevated in the transfectants with FRA3B integration after 5 d aphidicolin treatment, FISH analysis was performed to determine whether translocations, deletions, or insertions of pSV2neo also occurred with comparable frequency. To evaluate the sensitivity of FISH in detecting plasmid integration at various sites, 25 cells from each of the three transfectant cell clones were examined before the cells were treated with aphidicolin. At each site of integration, signal was observed in 72%–100% of the cells. As described above, after aphidicolin treatment, 20 cell clones were isolated from each of the three transfectant lines and 25 cells were analyzed from each of the 60 cell clones. A consistent pattern of hybridization was found within each clone; in clones showing a new location of hybridization, this was found in ≥80% of cells within the clone.

Table 1

Chromosome Aberrations of Human der(3) Induced after 5 d of Aphidicolin Treatment

	No. (%) of Aberrations							
	der(3)				Band 3p14			
Cell Line*	Deletions	Translocations	Insertions	Total	Deletions	Translocations	Insertions	Total
H3-4	2 (10)	3 (15)	1 (5)	6 (30)	2 (10)	2 (10)	1 (5)	5 (25)
TII4d	3 (15)	3 (15)	1 (5)	7 (35)	3 (15)	3 (15)	1 (5)	6 (35)
T16d	6 (30)	5 (25)	0	11 (55)	5 (25)	5 (25)	0	10 (50)
TII6b	4 (20)	7 (35)	0	11 (55)	4 (20)	7 (36)	0	11 (55)

Note. — Twenty-five cells were analyzed from each of 20 cell clones isolated from each cell line.

In addition, since signal is seen in the appropriate sites in the great majority of untreated cells, the complete absence of signal from a site in 25 cells analyzed is likely to represent a deletion of vector sequences rather than artifact.

In cell clones derived from the TI6d (fig. 1*D*) and TII6b (fig. 1*C*) lines, the position of pSV2neo signal corresponded closely to the gross chromosomal rearrangements observed (table 2). Thus, in TI6d-derived cell clones, pSV2neo was detected at FRA3B in 11 of 20 cell clones; pSV2neo had been translocated to chromosome 10 (p arm) in 1 (5%) of the cell clones and had been deleted in 8 (40%) of the cell clones. In one of the cell clones with a translocation affecting band p14 of chromosome 3, the signal remained on the der(3) (table 2). In cell clones from TII6b, pSV2neo was detected at FRA3B in 9 of 20 cell clones; pSV2neo had been translocated to hamster chromosome 9 in one cell clone and to hamster chromosome Z2 in another cell clone and had been deleted in nine (45%) of the cell clones. Although the deletion of pSV2neo signal was observed in all the cell clones with chromosomal deletions at FRA3B, pSV2neo sequences appear to be deleted in five of seven of the cell clones with chromosomal translocations involving FRA3B (table 2). The loss of vector sequences from the original site of integration as compared with the TII4d line was highly significant ($P < .003$) for both the TI6d and TII6b cell clones.

Detection of chromosome rearrangements involving hamster chromosomes 1 and mar2, by trypsin-Giemsa banding analysis in derived cell clones after 5 d aphidicolin treatment, revealed that the frequency was not as high as that observed at FRA3B (data not shown). However, in derived cell clones, evaluation of pSV2neo signal by FISH suggested that some deletions

Table 2

Comparison of the Chromosomal Changes and Position of pSV2neo at FRA3B in 20 Cell Clones Derived from Cell lines TI6d and TII6b

	TI6d		TII6b	
Cell Clone	Chromosome Changes	Presence of pSV2neo	Chromosome Changes	Presence of pSV2neo
1	None	+	None	+
2	None	+	None	+
3	None	+	None	+
4	None	+	None	+
5	None	+	None	+
6	None	+	None	+
7	None	+	None	+
8	None	+	None	+
9	None	+	None	+
10	None	+	del	−
11	del	−	del	−
12	del	−	del	−
13	del	−	del	−
14	del	−	*t	−
15	del	−	*t	−
16	t(3;**10**)(p14;**p2?1**)	der(**10**)	*t	−
17	*t	−	*t	−
18	*t	−	*t	−
19	*t	−	t(3;**9**)(p14;**p1?2**)	der(**9**)
20	*t	+	t(3;**Z2**)(p14;**q2?1**)	der(**Z2**)

Note.— + = Present; − = absent (pSV2neo sequences were not detected on the der(3) or at any other sites in the genome); del = deletion; and t = translocation. Identified hamster chromosomes involved in translocations are indicated in boldface type. Asterisks on translocations indicate unbalanced translocations in which the human der(3) is rearranged with unknown hamster chromosome material. The new position of pSV2neo is indicated in parenthesis. In TI6d derived cell clones 1–4 there are deletions of pSV2neo at hamster chromosome 1q26-31. In TII6b derived cell clones 1, 2, and 5 there are deletions of pSV2neo, and cell clone 9 has a translocation to hamster chromosome 8.

and translocations of pSV2neo were not detectable by conventional trypsin-Giemsa banding analysis (table 2). In TI6d, 16 of 20 cell clones retained signal at hamster chromosome 1 (q26-31), and in 4 of 20 cell clones, the signal was deleted. Only two of the cell clones with deleted pSV2neo signal showed detectable chromosomal translocations involving band q26-31 of hamster chromosome 1 (data not shown), whereas no deletion or other rearrangement was cytogenetically visible in the other two cell lines with loss of pSV2neo. In TII6b, 16 of 20 cell clones retained signal at mar2 (q11-13), and in one cell clone, pSV2neo signal had been translocated to hamster chromosome 5, and in three cell clones, deletion of signal had occurred. A detectable chromosomal deletion involving band q11-13 of hamster mar2 was found in only one cell clone (data not shown), while two cell clones with deleted signal had no cytogenetically apparent abnormalities at this band.

Discussion

Our studies demonstrate that there is a dramatic increase in structural chromosome rearrangements at FRA3B in the TI6d and TII6b lines (with specific pSV2neo integration at 3p14) after 24 h aphidicolin treatment, whereas only gaps and breaks were observed in the parental H3-4 cell line or in the TII4d cell line, the transfectant that did not have integration of pSV2neo at the FRA3B. The significant increase in the total number of chromosome aberrations in the FRA3B region suggests an increase in genomic instability at that site. Thus, 43%–67% of the total number of deletions and translocations observed in the TI6d and TII6b cell lines occurred at FRA3B, indicating that these genetic rearrangements contributed substantially to the overall increase in genomic alterations observed. After 5 d aphidicolin treatment, a less dramatic, but nonetheless significant, excess in the frequency of structural rearrangements at FRA3B was observed in cell clones derived from the lines with pSV2Neo integration at 3p14, in comparison with the lines without plasmid integration at that site. Deletions of pSV2neo also occurred at FRA3B; in some cases these sequences appeared to have been inserted into new chromosomal sites. A greater frequency of rearrangements at FRA3B in the TI6d and TII6b lines, in comparison with the lines without FRA3B integration, after both 24 h and 5 d aphidicolin treatment, supports the notion that integration of the plasmid at 3p14 resulted in increased genomic instability.

A significant increase in the frequency of chromosome aberrations was also observed at regions of pSV2neo integration in the hamster genome, specifically at bands q26-31 of chromosome 1 and at bands q11-13 of mar2. In cell clones after 5 d aphidicolin treatment, genetic rearrangements detected by FISH analysis had occurred at sites of pSV2neo integration into hamster bands. The TII4d transfectant cell line, with pSV2neo integrated at sites other than fragile sites, demonstrated a level of fragile-site expression, at both FRA3B and the two hamster fragile sites, that was not significantly different from that of the parental line (H3-4).

The causes for instability in specific regions of the genome is unknown. It seems unlikely that, in this case, it is due solely to specific sequences contained within the plasmid, since the sites of pSV2neo integration in the cell line TII4d show a much lower frequency of chromosome breakage and rearrangement. This effect may be caused by an interaction between pSV2-neo and specific sequences at the fragile site; an effect of the SV40 enhancer on transcription of host genes may further interfere with DNA synthesis in a region whose replication is already unusually sensitive to partial inhibition of DNA polymerase.

Murnane and Young (1989), among others, have identified specific sites of plasmid integration which are genetically "unstable"; significantly elevated levels of both homologous and nonhomologous recombination were observed in G418-resistant cell clones containing a transfected neogene. They performed Southern blot analysis of DNA isolated from spontaneous G418-resistant subclones of the cell line LM205 and demonstrated rearrangements in the region of integrated plasmid. Some investigators have suggested that the instability at some sites of plasmid integration is associated with repetitive cellular nucleotide sequences in proximity to plasmid sequences (Perucho et al. 1980). This hypothesis is compatible with a model proposed for the nature of fragile sites, i.e., that they represent repetitive DNA sequences (Laird et al. 1987). Thus, the sites of unstable vector integration studied by these investigators may in fact have been fragile sites. The reason(s) for the high rate of chromosome aberrations at these sites of integration is not known. Nonetheless, given both the increasing importance of introducing stable integrated sequences into mammalian cells and the possibility that similar naturally occurring hot spots for recombination could promote genomic instability, the characterization of DNA sequences at these sites will be of value.

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